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(54) Title: CANNABINOIDS SELECTIVE FOR THE CB2 RECEPTOR

(57) Abstract

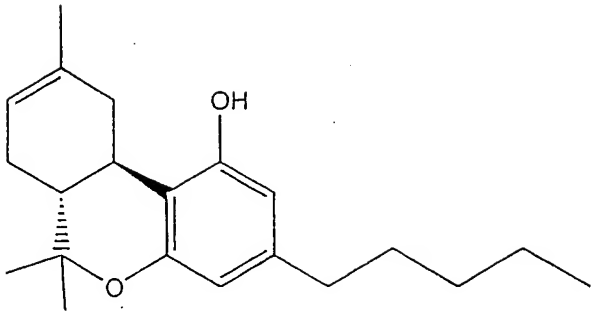
Disclosed are novel cannabinoids which are selective for the CB2 receptor. The novel cannabinoids comprise a substituted or unsubstituted tricyclic cannabinoid core and a substituted or unsubstituted C5–C8 carbocyclic ring, five to eight–membered heterocyclic ring or a seven to ten membered bicyclic ring system fused to the monohydroxylated phenyl ring of the cannabinoid core. Also disclosed are methods of suppressing the immune system in a subject by administering to the subject a (e.g. immunosuppressive amount) effective amount of a novel cannabinoid described above.

CANNABINOIDS SELECTIVE FOR THE CB2 RECEPTOR

BACKGROUND OF THE INVENTION

Δ^8-Tetrahydrocannabinol, the pyschoactive marijuana derived cannabinoid, binds to the CB1 receptor in the brain and to the CB2 receptor in the spleen. Activation of the CB2 receptor has been shown to result in suppression of the immune system (Mechoulam, *Cannabinoids as Therapeutic Agents*, CRC Press, Boca Raton, FL (1986)). Thus, drugs which selectively activate the CB2 receptor have great potential as immunomodulatory agents for preventing tissue rejection in organ transplant patients and as immunosuppressive agents for treating autoimmune associated diseases, (e.g., lupus erythematosus, rheumatoid arthritis, psoriasis, multiple sclerosis and inflammatory bowel diseases such as ulcerative colitis and Crohn's disease). CB2 receptor agonists also can be used as anti-inflammatory agents and as agents for suppressing peripheral and idiopathic pain.

Unfortunately, most known CB2 receptor agonists, including most cannabinoids, are non-selective in that they also stimulate the CB1 receptor. Activation of the



Delta-8-Tetrahydrocannabinol

Based on these results, novel compounds which are selective CB2 receptor agonists and the use of these compounds for modulating the immune system in a subject are disclosed.

One embodiment of the present invention is a compound comprising a substituted or unsubstituted tricyclic cannabinoid core. The cannabinoid core comprises a phenyl ring and a six-membered carbocyclic ring fused to a central pyran ring or to a central six-membered lactone ring (preferably a pryan ring). A substituted or unsubstituted C5-C8 carbocyclic ring, a five to eight-membered heterocyclic ring or a seven to ten membered bicyclic ring is fused to the phenyl ring. Also included are physiologically acceptable salts of the compound.

Another embodiment of the present invention is a method of suppressing the immune system in a subject. The method comprises administering to the subject a therapeutically effective amount of a compound

Figure 3 is a schematic showing two synthesis of (-)-Δ^8-tetrahydrocannabinol and cannabinol analogs with a bridged bicyclic side chains.

Figure 4 is a schematic showing a synthesis of (-)-Δ^8-tetrahydrocannabinol and cannabinol analogs with substituted cyclic side chains.

Figures 5A, 5B and 5C show the structure of a number of the novel compounds of the present invention.

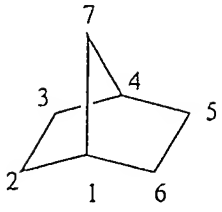
DETAILED DESCRIPTION OF THE INVENTION

Cannabinoids have a core tricyclic ring system in which a phenyl ring and a six membered ring are each fused to a central pyran ring or to a six-membered lactone ring (preferably a pyran ring). In addition, cannabinoids are able to induce characteristic physiological effects in mammals, including euphoria, delerium, drowsiness, hallucinations, weakness and/or hyporeflexia. The tricyclic core found in some cannabinoids is shown in Structural Formula (I). Other cannabinoids have the tricyclic core shown in Structural Formula (I), modified to include one or more double bonds in Ring A, for example, a double bond between carbons 8 and 9, between carbons 9 and 10 or between carbons 9 and 11. Yet other cannabinoids have the core structures described above, modified to include hydrogen, hydroxyl, hydroxymethyl, halogen (chloro, bromo, iodo and fluoro), methoxy, ethoxy, nitrile, nitro, halogenated methyl, halogenated ethyl, methoxymethyl, ethoxymethyl, nitromethyl, ethyl or $-CH_2CN$ group bonded to carbon 11 instead of a methyl group. In other cannabinoids, the hydroxyl group at position 1 of the core structure is replaced, for example, with -H,

five to eight-membered heterocyclic ring or a seven to ten membered bicyclic ring fused to positions two and three of the cannabinoid core.

Suitable substituents for a tricyclic cannabinoid core include groups which do not significantly diminish the ability of a cannabinoid to activate a cannabinoid receptor. Substitutions can occur at positions 2, 4, 6a-10a or at the three methyl groups. Substitution(s) at more than one position are possible. Substituents which do not significantly diminish the biological activity of cannabinoids are generally small, pharmacophoric groups. Examples include -H, -OH, $-OCH_3$, $-OCH_2CH_3$, halogen (e.g., chloro, bromo, iodo and fluoro), -CN, azido, isocyanate, isothiocyanate, $-NO_2$, $-CH_3$, $-C(halogen)_3$, $-CH_2OH$, $-CH_2OCH_3$, $-CH_2OCH_2CH_3$, $-CH_2(halogen)$, $-CH_2CN$, $-CH_2NO_2$, $-CH_2CH_3$ and $-CH_2C(halogen)_3$. Cannabinoids with other substituents can be prepared by modification of the synthetic procedures described in Example 1 and shown in Figures 2-4. For example, replacing the alcohol which reacts with compound 4 in Scheme 1 of Figure 2 with a suitably substituted analog results in the preparation of cannabinoids with a substituted cyclohexene ring or with substituents on one of the methyl groups attached to the pyran or cyclohexene ring. Other suitable substituents can be identified by testing modified cannabinoids in the *in vitro* CB1 or CB2 assays described in Example 2.

Two rings are fused when they share one single bond, one double bond or two adjacent ring atoms. For example, a cyclohexane ring fused to a phenyl ring forms a tetrahydronaphthalene group; a cyclopentane ring fused to a phenyl ring forms an indane group. In the present



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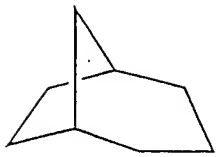

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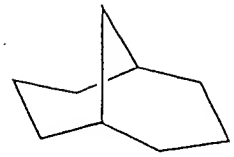

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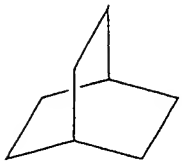

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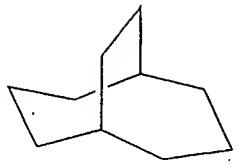


(VI)

The nomenclature for bicyclic ring systems indicates the number of rings atoms between bridgeheads. A "bridgehead" is an atom shared by both rings. For example, bicyclo 2.2.1. heptane, shown in Structural Formula (II), has two (C-2 and C-3), two (C-5 and C-6) and one (C-7) carbons between the bridgeheads (C-1 and C-4)

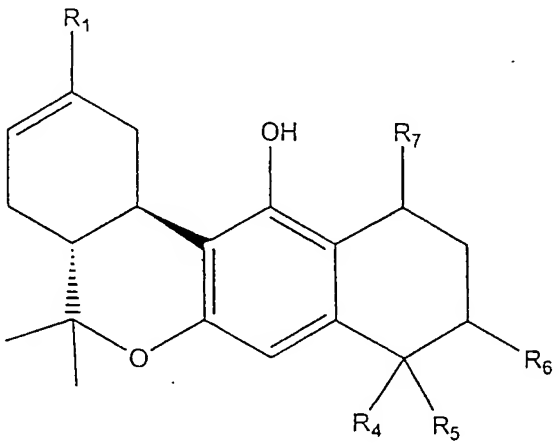
Suitable substituents for the fused carbocyclic, heterocyclic rings and bicyclic ring systems are generally C1-C8 alkyl groups, C1-C8 substituted alkyl groups or small, pharmacophoric groups. Examples of small pharmacophoric groups include, but are not limited to, -H, -OH, $-OCH_3$, $-OCH_2CH_3$, halogen (e.g., chloro,

X is $>C(CH_3)_2$ or -C=O. X is preferably $>C(CH_3)_2$.

R_1 is -H, -OH, $-OCH_3$, $-OCH_2CH_3$, halogen (chloro, bromo, iodo and fluoro), -CN, $-NO_2$, $-CH_3$, $-C(halogen)_3$, $-CH_2OH$, $-CH_2OCH_3$, $-CH_2OCH_2CH_3$, $-CH_2(halogen)$, $-CH_2CN$, $-CH_2NO_2$, $-CH_2CH_3$ or $-CH_2C(halogen)_3$. R_1 in Structural Formula (VII) is preferably -H or -OH.

R_2 and R_3, taken together with the carbon atoms to which they are bonded, form a substituted or unsubstituted C5-C8 monocyclic or C7-C10 bicyclic carbocyclic ring.

In a more preferred embodiment, the selective CB2 agonist of the present invention is represented by Formula (VIII):



(VIII)

R_1 is $-CH_3$ or $-CH_2OH$.

Specific examples of the compounds of the present invention are shown in Figures 1 and 5.

In another preferred embodiment, the selective CB2 agonist of the present invention is represented by Formula (VII), (VIII) or (IX), modified so that the hydroxyl group attached to the phenyl ring is replaced with an -H.

A "therapeutically effective amount" is the quantity of compound which results in immune system suppression in a subject after administration of the compound. Typically, a "therapeutically effective amount" of the compound ranges from about 10 mg/day to about 1000 mg/day, preferably from about 50 mg/day to about 500 mg/day. The specific dosage level of active ingredient will depend upon a number of factors, including, for example, biological activity of the particular preparation, age, body weight, sex and general health of the subject being treated.

As used herein, a "subject" refers to a human or animal. An "animal" refers to veterinary animals, such as dogs, cats, horses, and the like, and farm animals, such as cows, pigs, guinea pigs and the like.

The compounds of the present invention can be administered by a variety of known methods, including orally, rectally, or by parenteral routes (e.g., intramuscular, intravenous, subcutaneous, nasal or topical). The form in which the compounds are administered will be determined by the route of administration. Such forms include, but are not limited to capsular and tablet formulations (for oral and rectal administration), liquid formulations (for oral, intravenous, intramuscular or subcutaneous

The resulting mixture was stirred at room temperature overnight and then poured into saturated ammonium chloride solution. The product was extracted in diethyl ether, organic extracts were combined, dried and solvents removed by rotary evaporation. The crude product was dissolved in 30 mL of chloroform. About 10 mg of p-toluenesulfonic acid was added and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was washed with 10% sodium bicarbonate solution, dried, and the chloroform evaporated. The residue was chromatographed on silica gel (10% ethyl ether-petroleum ether) to afford 1.90 g (82%) of pure 2 as a colorless oil.

Compound 3. 1.20 g (3.95 mmol) of the alkene 2 was dissolved in 40 mL of absolute ethanol, 200 mg of 10% Pd on C catalyst was added and the solution was subjected to hydrogenation at room temperature and atmospheric pressure for 2 h. The catalyst was filtered off and the filtrate was rotary evaporated. The residue was purified on a small silica gel column to afford 1.11 g (92%) of compound 3.

Compound 4. A piece of potassium metal 105.5 mg (2.70 mmol) in 4 mL of dry THF was heated to reflux with vigorous stirring and then cooled quickly in an ice bath. To this potassium sand, a solution of 0.7 g (2.29 mmol) of compound 3 in 1 mL of THF was added in one portion under a blanket or argon. The red-color mixture was stirred at room temperature for 24 h and then terminated by addition of small amount of methanol

followed by water. After acidification, the reaction mixture was extracted with diethyl ether. Combined ether extracts were dried and ether evaporated to afford an oil which was chromatographed to give 260 mg (45%) of the pure product.

A solution of the above dimethyl ether (250 mg, 0.9 mmol) in dichoromethane was cooled in ice bath under argon and 1.26 mL of a 1 M solution of boron tribromide (2.26 mmol of BBr3) in dichloromethane was added dropwise. The reaction mixture was stirred at room temperature for 2 h and then quenched by cautious addition of water. The organic layer was separated, washed with 10% sodium bicarbonate and dried. Rotary evaporation gave a crude product which was chromatographed on silica gel (50% ethyl ether-petroleum ether) to afford 190 mg (85%) of resorcinol 4.

Compound 5 & 6. 7 mg of p-toluenesulfonic acid was added to the solution of resorcinol 4 (63.3 mg, 0.25 mmol) and 43.6 mg of cis/trans-p-menthedienol (0.28 mmol) in 2.5 mL of chloroform, and the mixture was refluxed for 45 min. The solution was cooled to room temperature, washed with 10% sodium bicarbonate, dried, and evaporated. The residue was chromatographed on silica gel (7% ethyl ether-petroleum ether) to afford 35 mg of tetrahydrocannabinol 5 and 7 mg of tetrahydrocannabinol 6 (combined yield 63%).

Compound 7. A solution of potassium bis(trimethysilyl)amide (2.03 mmol in 5 mL of dry THF) was cooled to -78 °C under argon and a solution of 0.4 g of compound 1 (1.69 mmol) in 1 mL of dry THF was added

Compound 9. Procedure used is similar to the one described for the preparation of compound 5. Starting from 61.6 mg (0.26 mmol) of the resorcinol 8, 59.2 mg (62%) of tetrahydrocannabinol 9 was obtained.

Compound 10. The mixture of 6.50 g of 2,5-dimethoxy phenol (42 mmol) and 7.70 g of (1S, 2S, 3S, 5R)-(+)-isopinocampheol (50 mmol) in 200 ml of 70% methanesulfonic acid was heated and stirred in a 70 °C oil bath for 24 hr. After cooling to room temperature, the reaction mixture was poured onto ice and extracted with methylene chloride. The extracts were washed with water and saturated with sodium bicarbonate solution, dried with sodium sulfate. Removal of solvent provided 15.0 g of brown oil crude. The crude was subjective to multiple column chromatography purification with the elution solvent of the mixture of hexane, methylene chloride and ethyl acetate (5: 5: 1). The component of Rf value of 0.52 was collected.

Compounds 11 to 13

These three compounds were prepared by the methods described in Dominiami *et al.*, *J. Org. Chem.* 42:344 (1977), the entire teachings of which are incorporated herein by reference.

Compounds 14 and 15

The mixture of 1 mmol of 13, 3 mmol trans-p-mentha-2, 8-dien-1-ol and 36 mg of p-toluenesulfonic acid monohydrate in 10 ml of chloroform was stirred and heated in a 70°C oil bath for 3 hours. Then the reaction temperature was lowered to room temperature.

were diced with a razor blade and homogenized in 0.32 M sucrose, pH 7.4. The resulting suspension was spun at 400 x g at 4°C. The supernatant was decanted and layered over 1.2 M sucrose in TME buffer (25 mM Tris base, 5 mM $MgCl_2$ 1 mMEDTA, pH 7.4) and spun at 109,000 x g. The interface containing plasma membrane protein was collected, pooled and layered over 0.8 M sucrose in TME, pH 7.4. The pellet was carefully resuspended in TME, pH 7.4 and the total protein content was assayed by the method of Markwell *et al.*, *Anal. Biochem.* 87:206 (1978), the entire teachings of which are incorporated herein by reference. Protein was aliquotted, frozen under liquid nitrogen and stored at -80°C until use.

Approximately 30μg of tissue was incubated in silanized 96 well microtiter plate with TME containing 0.1% essentially fatty acid free bovine serum albumin (BSA), 0.8 nM [H^3]CP-55,940 and various concentrations of the test compound in a final volume of 200 μL. Assays were incubated at 30°C for 1 hour. The samples were filtered using Packard Filtermate 196 and Whatman GF/C Filterplates and washed with wash buffer (TME) containing 0.5% BSA. Radioactivity was detected using MicroScint 20 scintillation cocktail added directly to the dried filterplates, and the filterplates were counted using a Packard Instruments Top-Count. Nonspecific binding was assessed using 100 nM CP-55,940. Data collected from three independent experiments performed with duplicate determinations were normalized between 100% and 0% specific binding for [H^3]CP-55,940, determined using buffer and 100 nM CP-55,940. The normalized data was analyzed using a 4 parameter

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

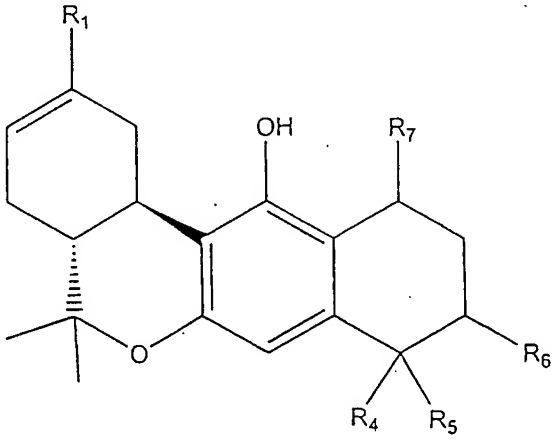
wherein Ring A has zero to three endocyclic double bonds;

X is $>C(CH_3)_2$ or -C=O;

R_1 is -H, -OH, $-OCH_3$, $-OCH_2CH_3$, halogen, -CN, $-NO_2$, $-CH_3$, $-C(halogen)_3$, $-CH_2OH$, $-CH_2OCH_3$, $-CH_2OCH_2CH_3$, $-CH_2(halogen)$, $-CH_2CN$, $-CH_2NO_2$, $-CH_2CH_3$ or $-CH_2C(halogen)_3$; and

R_2 and R_3, taken together with the carbon atoms to which they are bonded, form a substituted or unsubstituted C5-C7 monocyclic or a C7-C10 bicyclic carbocyclic ring.

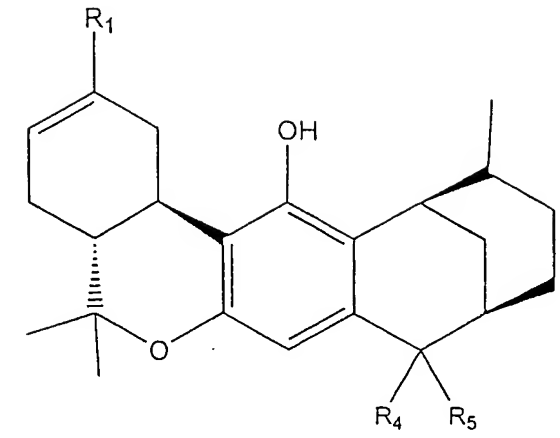
3. The compound of Claim 2 wherein the compound is represented by the following structural formula:



;

wherein:

R_1 is $-CH_3$ or $-CH_2OH$;



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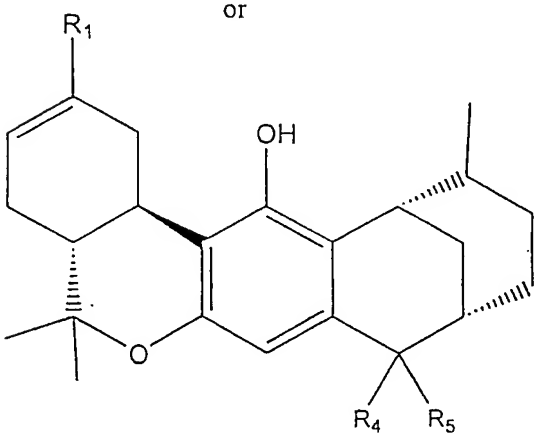


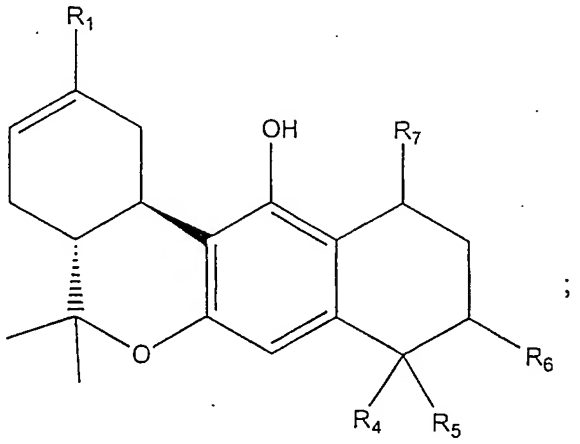
7. A method of suppressing the immune system in a subject comprising administering to the subject a therapeutically effective amount of a compound

X is $>C(CH_3)_2$ or -C=O;

R_1 is -H, -OH, $-OCH_3$, $-OCH_2CH_3$, halogen, -CN, $-NO_2$, $-CH_3$, $-C(halogen)_3$, $-CH_2OH$, $-CH_2OCH_3$, $-CH_2OCH_2CH_3$, $-CH_2(halogen)$, $-CH_2CN$, $-CH_2NO_2$, $-CH_2CH_3$ or $-CH_2C(halogen)_3$; and

R_2 and R_3, taken together, form a substituted or unsubstituted C5-C7 monocyclic or C7-C10 bicyclic carbocyclic ring.

9. The method of Claim 8 wherein the compound is represented by the following structural formula:

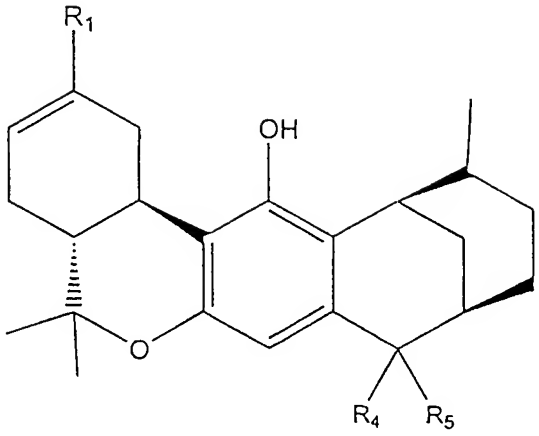


;

wherein:

R_1 is $-CH_3$ or $-CH_2OH$;

R_4-R_7 are independently -H or a C1 to C10 straight chained substituted or unsubstituted alkyl group; and



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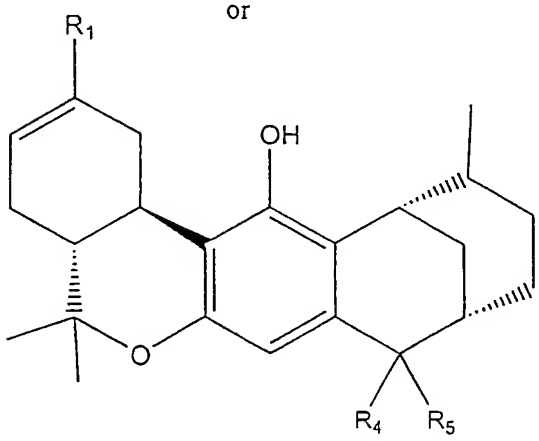
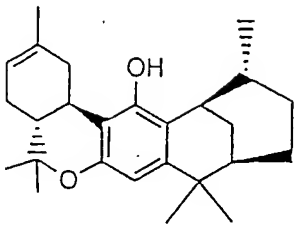


13. A substituted or unsusbstituted cannabinoid having a side chain comprising a substituted or unsubstituted C5-C8 carbocyclic ring, a five to



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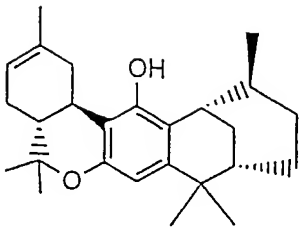

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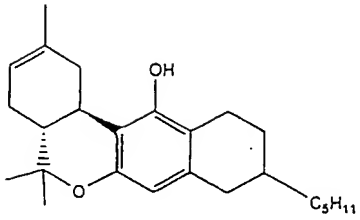

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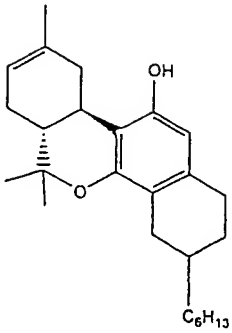

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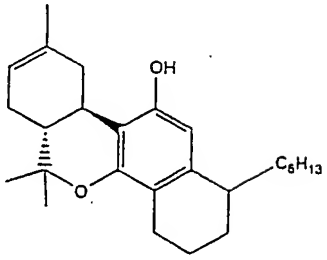

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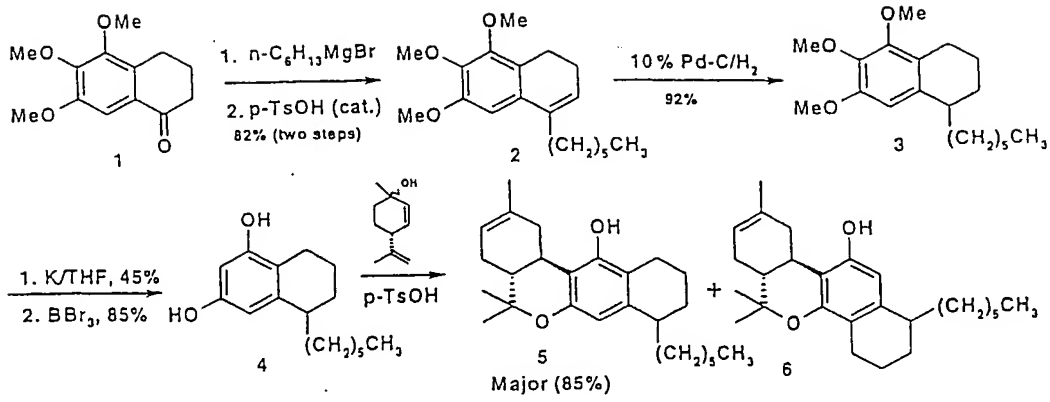
FIGURE 1

Scheme 1



Scheme 2

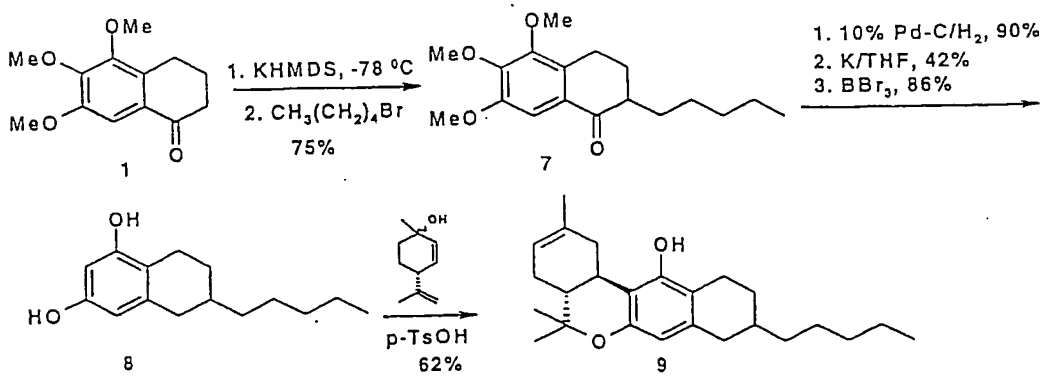


FIGURE 2

Scheme 3

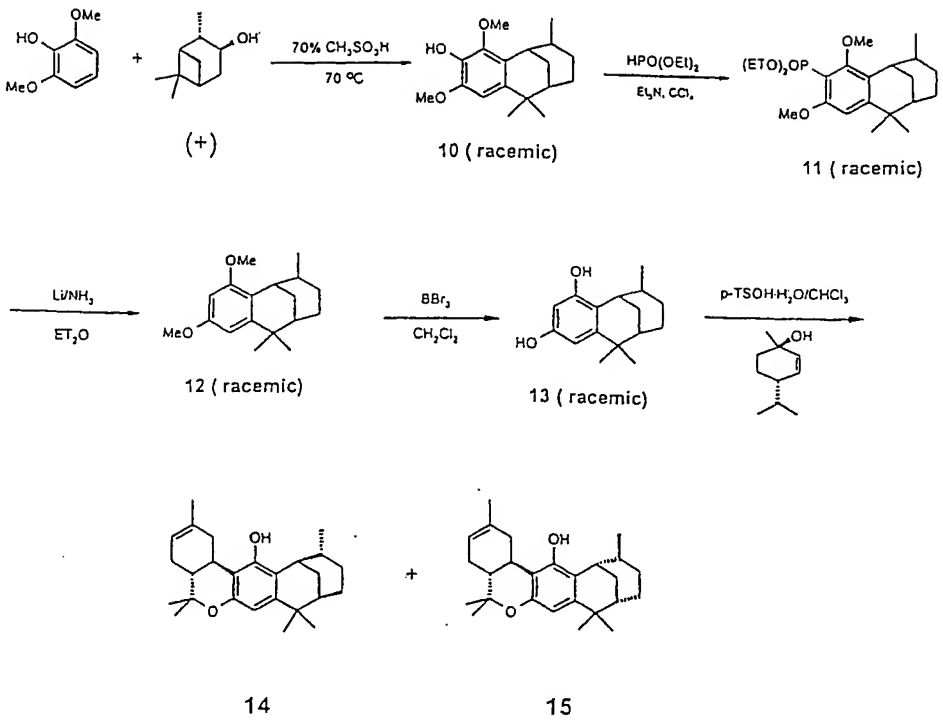


Figure 3

Scheme 4

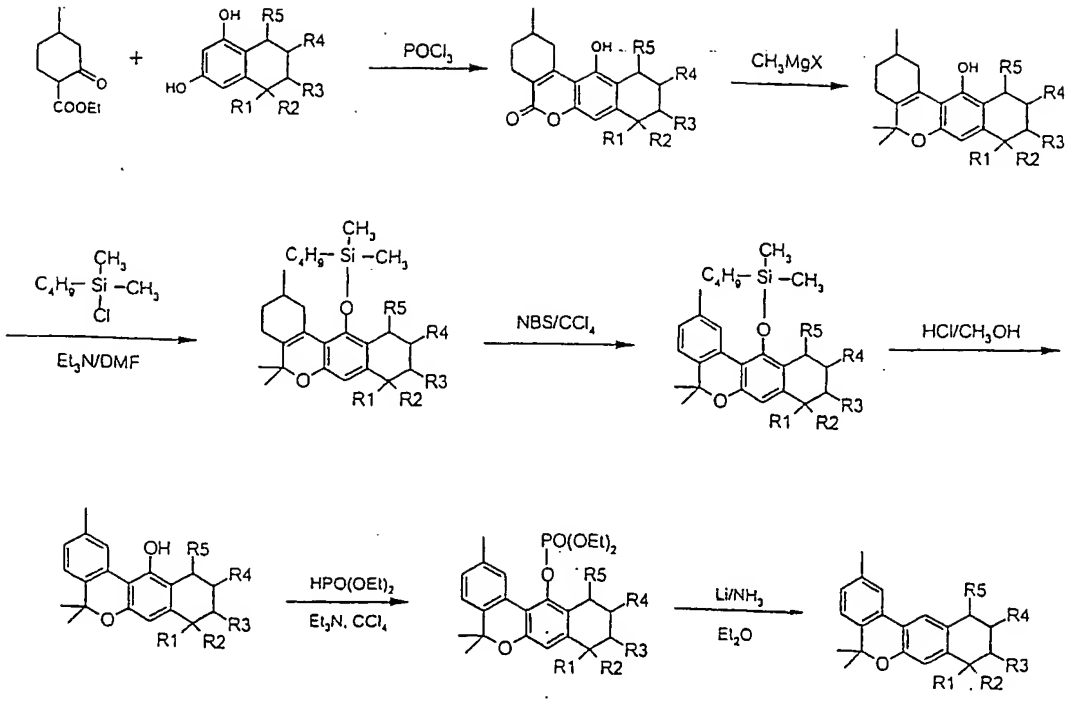


Figure 4

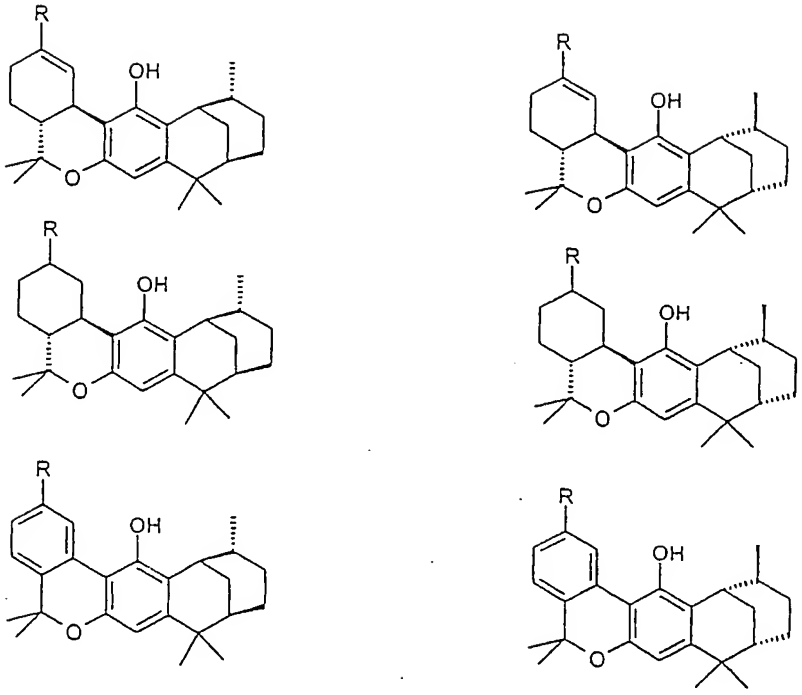


FIGURE 5A

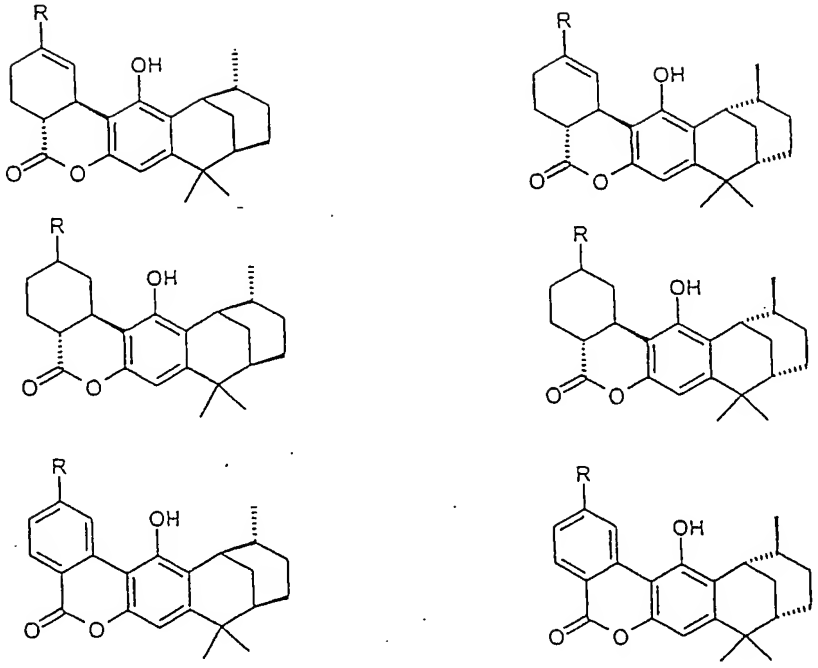
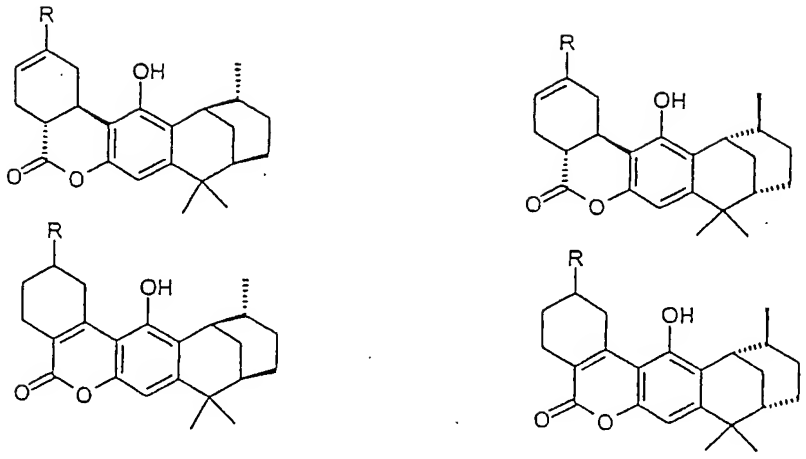


FIGURE 5B



R = $-CH_3$ or $-CH_2OH$

FIGURE 5C